# Antibacterial Activity against *E. coli* O157:H7, Physical Properties, and Storage Stability of Novel Carvacrol-Containing Edible Tomato Films

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**ABSTRACT: Edible films containing plant antimicrobials are gaining importance as potential treatment to extend product shelf life and reduce risk of pathogen growth on contaminated food surfaces. The main objective of the present study was to evaluate the antimicrobial activities, storage stabilities, and physical–chemical–mechanica1 properties of novel edible films made from tomatoes containing carvacrol, the main constituent of oregano oil. The antimicrobial activities against *E. coli* O157:H7 and the stability of carvacrol were evaluated during the preparation and storage of tomato-based films made by 2 different casting methods, continuous casting and batch casting. Antimicrobial assays of tomato films indicated that optimum antimicrobial effects occurred with carvacrol levels of approximately 0.75% added to tomato purees before film preparation. HPLC analysis of the films indicated that the carvacrol concentrations and bactericidal effect of the films remained unchanged over the storage period of up to 98 d at 5 and 25 °C. Carvacrol addition to the tomato puree used to prepare the films increased water vapor permeability of tomato films. The continuous method for casting of the films appears more suitable for large-scale use than the batch method. This 1st report on tomato-based edible antimicrobial tomato films suggests that these films have the potential to prevent adverse effects of contaminated food and promote human health associated with the consumption of tomatoes.**

**Keywords: antibacterial tomato films, carvacrol, *E. coli* 0157:H7, HPLC, physical properties, storage stability**



## Introduction

Major outbreaks involving fresh produce have been associated with common foodborne pathogens such as *Salmonella*, *Listeria monocytogenes*, *Shigella* spp., and *Escherichia coli* O157:H7 (Beuchat 1996; Smith and Fratamico 2005). In September 1997, an EPA Scientific Advisory Panel specifically identified *Salmonella, L. monocytogenes*, and *E. coli O157:H7* as pathogens of public health concern on produce (Brackett 1999). In previous publications, we reported on the antibacterial activities of carvacrol, a major constituent of oregano and thyme plant essential oils, against pathogenic bacteria in buffers (Friedman and others 2002; Ravishankar and others 2008a), apple juices (Friedman and others 2004), wines (Friedman and others 2006), and in ground beef, chicken, and turkey (Juneja and others 2006; Juneja and Friedman 2008). In related studies, we reported that edible apple-based films containing low levels of plant essential oils and their major constituents induced reduction of the foodborne pathogen *E. coli* O157:H7 (Rojas-Graü and others 2006, 2007; Du and others 2008). Other studies showed that *E. coli* O157:H7 is more acid resistant and more likely to survive in tomato products (Eribo and Ashenafi 2003) and that this pathogen is more resistant to carvacrol *in vitro* than are *Salmonella enterica*, *Campylobacter jejuni*, and *Listeria monocytogenes* (Friedman and others 2002). It is also relevant to note that carvacrol is considered a volatile molecule. The tendency of volatile molecules to pass in the vapor phase is related to their interaction with water and other solvents/solutes in the edible films matrices, as well as to changes in storage temperature (Gardini and others 1997; Voilley and Souchon 2006). Depletion of volatile compounds during storage can be substantial, leading to reduction of antibacterial activity (Debeaufort and others 2002).

In addition to its flavor properties, tomatoes are reported to possess numerous beneficial nutritional and bioactive components that may also benefit human health. These include the nutrients vitamin A, vitamin C, iron, and potassium; nonnutritive digestible and indigestible dietary fiber; the antioxidative compounds lycopene, $\beta$-carotene, and lutein (Frusciante and others 2007; Dorais and others 2008); and the cholesterol lowering (Friedman and others 2000a, 2000b) and immune system enhancing glycoalkaloids tomatine and dehydrotomatine (Morrow and others 2004). Consumption of tomatoes, tomato products, and isolated bioactive tomato ingredients is reported to be associated with lowered risk of cancer (Friedman and others 2007), heart disease (Willcox and others 2003), diabetes (Bose and Agrawal 2007), and hypertension (Engelhard and others 2006).

These considerations suggest that edible tomato films containing antimicrobials may have multiple benefits. These include protection of food against contamination by pathogenic microorganisms and nutritional and health benefits associated with the consumption of the above-mentioned tomato ingredients that may be present in the films.

Because of the cited potential inherent advantages of tomato-based films for human health, the major objectives of the present study were to (1) determine the physical–chemical properties and storage stability of carvacrol-containing tomato-based films with

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the aid of HPLC and physical–chemical–mechanical assays; (2) to evaluate the antimicrobial activities of the films against *E. coli* O157:H7; and (3) to define the relationship between carvacrol levels of films and antimicrobial effectiveness. To our knowledge, this is the 1st report on tomato-based antimicrobial films (Joerger 2007; Ponce and others 2008).

## Materials and Methods

### Source of bacteria

The Food and Drug Administration (FDA) provided *E. coli* O157:H7 bacteria (our strain designation RM1484; original designation SEA13B88 (Friedman and others 2002).

### Preparation of tomato films

**Preparation of the tomato film forming solution.** Hot break tomato puree (31 °Brix, The Morning Star Packing Co., Los Banos, Calif., U.S.A.) was the primary ingredient in all tomato-based film forming solutions (pastes). High methoxyl pectin 1400 (TIC Gums, Belcamp, Md., U.S.A.) was added to increase film strength and facilitate release from cast surfaces. Carvacrol was donated by Millenium Chemical Co. (Jacksonville, Fla., U.S.A.).

The tomato paste (30%, w/w; 300 g of 31 °Brix tomato puree plus 700 g of 3% [w/w] pectin solution) was combined in a mixer bowl, and mixed on slow speed for 30 min. Carvacrol was then incorporated into the tomato puree solutions at the following concentrations: 0 (control), 0.5, 0.75, 1.0, and 1.5 (w/w). These solutions were homogenized for 2.5 min at 20000 rpm using a Polytron 3000 homogenizer (Kinematica, Littau, Switzerland). Each homogenate was degassed under vacuum for 15 min and then used for casting the films.

**Film casting.** Two methods were used to cast films: continuous casting and batch casting. Edible tomato films were continuous cast with a Mathis Labcoater unit (Werner Mathis AG, Zürich, Switzerland) by spreading 41 mils (1 mil = 0.001 inches) thickness tomato solution on a Mylar sheet conveyor moving at 0.11 m/min. The film was first partially dried by an infrared heater adjusted to 0% and 90% infrared energy emission at top and bottom, respectively. Residence time in this stage was 4 min to a maximum temperature of 100 °C. Next the convective heating stage was controlled at 132 °C and 1500 m/min air velocity. Residence time in this stage was 8 min. Casting of films in the Labcoater takes 12 min. Batch cast films were produced on the bench. They were made using a 45 mil gap drawdown bar to spread the tomato solutions on a flat Mylar sheet placed on a 29 × 29 cm square glass plate, which was then dried overnight at room temperature (20 to 25 °C) in a sterile biohood.

### Physical–chemical–mechanical properties of films

**Water vapor permeability (WVP).** The Gravimetric Modified Cup Method (McHugh and others 1993) based on standard method ASTM E96-80 (ASTM 1980) was used to determine WVP. A cabinet with a variable speed fan was used to test film WVP. Cabinet temperature was maintained at 25 ± 1 °C. Fan speeds were set to achieve air velocities of 80 m/min to ensure uniform relative humidity throughout the cabinets. Cabinets were pre-equilibrated to 0% relative humidity (RH) using anhydrous calcium sulfate (W.A. Hammond Drierite, Xenia, Ohio, U.S.A.). Circular test cups made from polymethylmethacrylate (Plexiglas™) were used. A film was sealed to the cup base with a ring containing a 19.6 $cm^2$ opening using 4 screws symmetrically located around the cup circumference. Both sides of the cup contacting the film were coated with silicon sealant. Distilled water (6 mL) was placed in the bottom of the test cups to expose the film to a high percentage RH inside the test cups. Test cups holding films were then inserted into the pre-equilibrated 0% RH desiccator cabinets. Steady state of water vapor transmission rate was achieved within 2 h. Each cup was weighed 8 times at 2-h intervals. Eight replicates of each film were tested. RHs at the film undersides and WVPs were calculated using the WVP correction method (McHugh and others 1993). Multiplying the steady-state water vapor transmission rate by the average film thickness and dividing by the water vapor partial pressure difference across the films calculated the WVP of the films:

$$\text{WVP} = \frac{(\text{WVTR})(\text{thickness})}{(p_{A1} - p_{A2})} \quad (1)$$

where WVTR = water vapor transmission rate and $p_{A1}$ and $p_{A2}$ = water vapor partial pressure on film interfaces inside and outside the cup, respectively. Units for WVP were g mm/kPa h $m^2$.

**Tensile properties.** Standard method ASTM D882-97 (ASTM 1997) was used to measure tensile properties of films. Films were cut into strips with a test dimension of 165 × 19 mm according to standard method ASTM D638-02a (ASTM 2002). All films were conditioned for 48 h at 23 ± 2 °C and 33% ± 2% RH before testing using a saturated solution of magnesium nitrate (Fisher Scientific, Fair Lawn, N.J., U.S.A.). The ends of the equilibrated strips were mounted and clamped with pneumatic grips on an Instron Model 55R4502 Universal Testing Machine (Instron, Canton, Mass., U.S.A.) with a 100 N load cell. The initial gauge length was set to 50 mm and films were stretched using a crosshead speed of 7.5 mm/min. Tensile properties were calculated from the plot of stress (tensile force/initial cross-sectional area) compared with strain (extension as a fraction of original length), using Series IX Automated Materials Testing System Software (Instron). Fifteen specimens of each film were evaluated.

**Film color.** Color of films under a Konica Minolta standard white reflector plate was measured with a Konica Minolta spectrophotometer (Model CM508D, Konica Minolta Inc., Ramsey, N.J., U.S.A.) using the CIE $L^*$, $a^*$, $b^*$ coordinates. Illuminant D65 and 10°-observer angle were used. The instrument was calibrated using a Minolta standard white reflector plate. Eleven films were evaluated for each carvacrol concentration. Three readings were made in each replicate by changing the position of the Chroma Meter over the film.

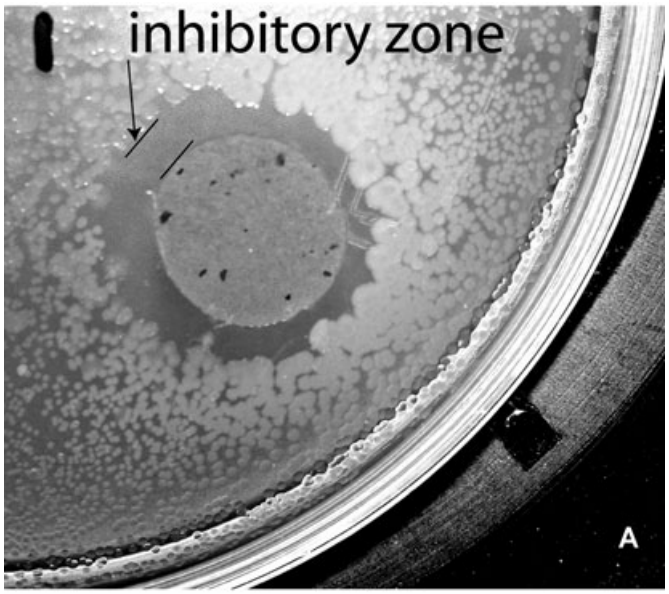
### Antimicrobial properties of films against *E. coli* O157:H7

**Storage studies.** Continuous and batch cast tomato films were shaped into 50-mm-dia discs by cutting with a razor blade around the edge of a watch glass over the film and stored without covering on meshed plastic shelves in a closed system in Forma Scientific refrigerated incubators (Mallinckrodt Inc., Marietta, Ohio, U.S.A.) at 5 °C and 34% RH and at 25 °C and 33% RH. The stored films were sampled at day 1 and weekly for 14 wk (98 d). Five film discs were taken out for testing on each sampling time. Stability of carvacrol in tomato films was determined by extracting the carvacrol from the films and then analyzing for carvacrol content by HPLC as described below. The antimicrobial activities of the stored films were also determined concurrently as described subsequently.

**Film discs for microbiology and HPLC studies.** For microbiology studies, each 50-mm film disc was further cut into 12-mm-dia discs using a sterilized cork borer. The film discs were stored on layers of aluminum foil in sealed, sterilized glass containers until used. The weight and thickness of films used for microbial testing were measured with an analytical balance and a micrometer, respectively.

For HPLC studies, three 50-mm-dia film discs were covered in folded aluminum foil, sealed in plastic bags, and stored under refrigeration until testing, within 24 h.

**Antimicrobial assay of *E. coli* O157:H7.** Frozen cultures of *E. coli* O157:H7 strain RM1484 from our bacterial strain collection was streaked on tryptic soy agar (TSA, tryptic soy broth and 1.5% granulated agar, Difco, Becton, Dickinson & Co., Sparks, Md., U.S.A.) and then incubated at 37 °C for 24 h. One isolated colony was restreaked on TSA and then incubated at 37 °C for 24 h. One isolated colony was then inoculated into a tube with 5 mL trypticase soy broth (TSB, Difco), and incubated at 37 °C for 24 h with agitation. The microbial broth was serially diluted (10×) in 0.1% peptone + 0.85% NaCl. Then, 0.1 mL of $10^5$ colony forming units (CFU)/mL of *E. coli* O157:H7 was plated onto each of 6 MacConkey-Sorbitol Agar (MSA, Difco) plates. The bacteria inoculum was spread evenly throughout each plate and let to dry for 5 min in a biosafety hood.



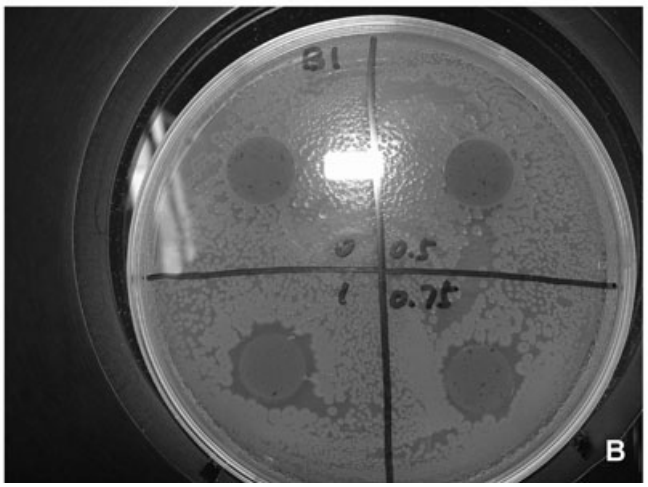




**Figure 1—(A) Inhibitory zone (antimicrobial effect) of *E. coli* O157:H7 growth on a bacterial plate induced by a carvacrol-containing tomato film measured with a digital caliper. (B) Inhibitory zones of *E. coli* O157:H7 growth around films with different concentrations of carvacrol (0% to 1%) added to tomato puree solutions used to prepare the films.**

Each agar plate was divided into 4 equal sectors and labeled with the different carvacrol concentrations. On the center of each area, one aseptically cut 12-mm-dia edible film disc was deposited over the inoculated agar with the film's shiny side placed down. The 3 shortest widths of the inhibition zone (Figure 1A) around the film disc (colony free perimeter) were measured with a digital caliper (Neiko Tools, Ontario, Calif., U.S.A.) after 24 and 48 h of incubation at 35 °C, respectively. The inhibition area was then calculated. The inhibition area of 6 disks per treatment was used for statistical analysis.

**Measurement of carvacrol in films by HPLC.** Each film was weighed (approximately 230 mg) and homogenized in an Omni Intl. Homogenizer (Gainesville, Va., U.S.A.) in 10 mL 50% ethanol (prepared from 95% ethanol, ACS/USP grade) for 5 min on low speed with a blade attachment, then for 5 min on high speed with a generator probe. The extract was filtered through a 0.45-$\mu$m nylon membrane (Sigma, St. Louis, Mo., U.S.A.) and injected directly into the HPLC column.

The HPLC system consisted of a Beckman 110B pump, a Thermo Separation Products AS3500 autosampler (loop size 100 $\mu$L), and a UV 3000HR-scanning detector with both deuterium and tungsten lamps. Thermo Separation Products PC1000 System Software controlled the system. The following conditions were used: a Supelco LC-ABZ column was used (250 × 4.6 mm plus a 2-cm precolumn); the particle size of the column packing was 5 $\mu$m; the eluent consisted of 50% acetonitrile, 50 mM ammonium phosphate, and 0.05% phosphoric acid, pH 3.1. The eluent was degassed once before use. The flow rate of the pump was 1 mL/min and the sample volume injected was 20 $\mu$L. Absorbance was monitored at 200 nm. Figure 2 shows a typical HPLC chromatogram.

## Statistical analysis

Data were analyzed by 2 and 1-way analysis of variance (ANOVA) using Minitab version 13.31 software (Minitab Inc., State College, Pa., U.S.A.). Tukey test was used to determine the difference at 5% significance level.

# Results and Discussion

## Physical–chemical properties of tomato films

**Water vapor permeability (WVP).** Based on 2-way ANOVA, WVP was significantly higher ($P < 0.05$) for batch-cast films than for continuous-cast films (Table 1). Higher casting temperatures used while making the continuous-cast films in the Labcoater increased rates of both carvacrol and water evaporation and made the films thinner and denser, probably reducing interstitial spaces for molecular diffusion. Because all the films were stored in an

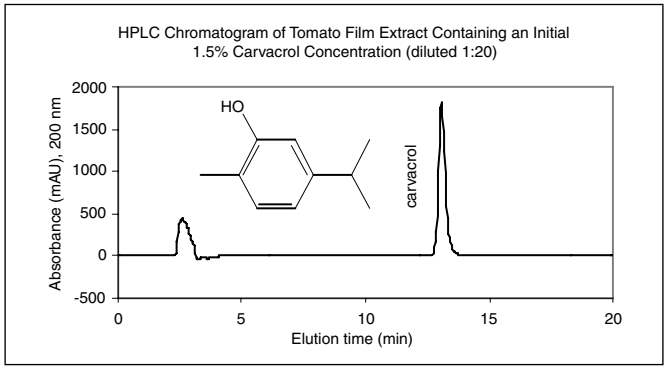



**Figure 2—HPLC chromatogram of a tomato film extract containing an initial 1.5% carvacrol in the tomato puree used to prepare the films.**

**Table 1—Effect of carvacrol concentration (% w/w) on water vapor permeability (WVP) of tomato puree edible films.[a,b]**

| Casting method | Carvacrol concentration (% w/w) | Film thickness (mm) | Relative humidity inside cup (%RH) | Water vapor permeability (WVP) (g-mm/kPa-h-m$^2$) |
|---|---|---|---|---|
| Batch-cast | 0 | $0.108 \pm 0.006^{a}$ | $81.5 \pm 0.9^{a}$ | $2.44 \pm 0.18^{a}$ |
| | 0.5 | $0.119 \pm 0.005^{b}$ | $83.2 \pm 0.6^{b}$ | $2.60 \pm 0.12^{ab}$ |
| | 1.0 | $0.126 \pm 0.003^{c}$ | $82.4 \pm 0.7^{ab}$ | $2.68 \pm 0.14^{b}$ |
| | 1.5 | $0.119 \pm 0.004^{b}$ | $82.0 \pm 0.8^{a}$ | $2.61 \pm 0.14^{ab}$ |
| Continuous-cast | 0 | $0.104 \pm 0.004^{AB}$ | $84.6 \pm 0.6^{BC}$ | $2.20 \pm 0.15^{NS}$ |
| | 0.5 | $0.103 \pm 0.003^{A}$ | $85.1 \pm 0.7^{C}$ | $2.11 \pm 0.17^{NS}$ |
| | 1.0 | $0.105 \pm 0.005^{AB}$ | $83.8 \pm 0.6^{B}$ | $2.18 \pm 0.14^{NS}$ |
| | 1.5 | $0.109 \pm 0.004^{B}$ | $82.7 \pm 0.7^{A}$ | $2.28 \pm 0.19^{NS}$ |

[a]Data reported are mean values ± standard deviations. [a,b,c;A,B,C]Means in column with different letters are significantly different at $P < 0.05$ for each of the casting methods.
[b]Relative humidity at the inner surface and WVP values were corrected for stagnant air effects using the WVP correction method (McHugh and others 1993).
[NS]Not significant differences between films.

**Table 2—Effect of carvacrol concentration (% w/w) on the tensile properties of tomato puree edible films.[a]**

| Casting method | Carvacrol concentration (% w/w) | Tensile strength (MPa) | Modulus (MPa) | Elongation (%) |
|---|---|---|---|---|
| Batch-cast | 0 | $11.4 \pm 1.5^{b}$ | $248.1 \pm 45.9^{b}$ | $11.2 \pm 2.0^{ab}$ |
| | 0.5 | $12.9 \pm 1.1^{c}$ | $310.3 \pm 41.1^{c}$ | $10.7 \pm 1.6^{ab}$ |
| | 1.0 | $11.1 \pm 1.3^{b}$ | $259.9 \pm 58.3^{b}$ | $9.5 \pm 2.4^{a}$ |
| | 1.5 | $8.9 \pm 0.7^{a}$ | $187.2 \pm 32.3^{a}$ | $11.6 \pm 2.2^{b}$ |
| Continuous-cast | 0 | $13.7 \pm 1.8^{BC}$ | $316.9 \pm 40.8^{A}$ | $9.6 \pm 1.4^{B}$ |
| | 0.5 | $14.8 \pm 2.2^{C}$ | $465.2 \pm 82.8^{C}$ | $7.0 \pm 1.4^{A}$ |
| | 1.0 | $12.2 \pm 2.3^{B}$ | $401.2 \pm 71.5^{B}$ | $6.0 \pm 1.3^{A}$ |
| | 1.5 | $10.4 \pm 1.3^{A}$ | $259.1 \pm 49.4^{A}$ | $8.6 \pm 1.1^{B}$ |

[a]Same footnote as in Table 1.

**Table 3—Effect of carvacrol concentration (% w/w) on color parameters of tomato puree edible films.[a]**

| Casting method | Carvacrol concentration (% w/w) | $L^*$ | $a^*$ | $b^*$ |
|---|---|---|---|---|
| Batch-cast | 0 | $59.6 \pm 2.2^{c}$ | $27.0 \pm 2.3^{a}$ | $43.5 \pm 0.8b^{c}$ |
| | 0.5 | $57.8 \pm 1.8^{b}$ | $27.4 \pm 1.7^{a}$ | $44.2 \pm 1.1^{c}$ |
| | 1.0 | $54.9 \pm 1.1^{a}$ | $29.2 \pm 1.0^{b}$ | $42.5 \pm 0.6^{a}$ |
| | 1.5 | $55.4 \pm 1.2^{a}$ | $29.1 \pm 1.1^{b}$ | $43.0 \pm 0.6^{ab}$ |
| Continuous-cast | 0 | $56.4 \pm 0.9^{AB}$ | $28.9 \pm 0.8^{B}$ | $44.8 \pm 1.1^{A}$ |
| | 0.5 | $57.3 \pm 0.9^{BC}$ | $26.1 \pm 0.7^{A}$ | $46.3 \pm 1.5^{AB}$ |
| | 1.0 | $58.1 \pm 0.8^{C}$ | $26.9 \pm 0.7^{A}$ | $48.2 \pm 1.1^{BC}$ |
| | 1.5 | $55.9 \pm 1.1^{A}$ | $29.4 \pm 0.9^{B}$ | $48.9 \pm 2.3^{C}$ |

[a]Same footnote as in Table 1.
[NS]Not significant differences between films.

incubator at constant temperature and humidity, moisture was not measured. The batch-cast tomato films were less dense than the continuous-cast tomato films, as evidenced by the following calculated respective average densities ($n = 84$): $1.189 \pm 0.105$ g/cm$^3$ and $1.242 \pm 0.092$ g/cm$^3$ ($P < 0.001$). Density was calculated from the weight and thickness of 12-mm-dia films. Storage temperature did not affect density of the tomato films.

WVP was increased significantly ($P < 0.05$) when carvacrol was added to the batch-cast tomato films made in the biohood. Compared to control tomato films without carvacrol, addition of carvacrol to tomato film solutions at concentrations 0.5% to 1.5% increased WVP of batch-cast films. This effect was not evident in continuous-cast films. The effect may be related to increased evaporation of carvacrol and increased film density due to the higher temperatures used in this casting method.

**Tensile properties.** Tensile strength, elongation, and elastic modulus are parameters that relate mechanical properties of films to their chemical structures (McHugh and Krochta 1994). The addition of carvacrol to tomato films decreased tensile strength. Batch-cast films had lower tensile strength than continuous-cast tomato films (Table 2). The data also show that (1) film modulus decreased as carvacrol concentration was increased; (2) batch-cast tomato films had lower modulus values than continuous-cast tomato films; (3) elongation [film's stretchability before breakage (Krochta and De Mulder-Johnston 1997)] of films prepared by batch casting was significantly higher than of those prepared by continuous casting ($P < 0.05$); and (4) added carvacrol reduced slightly the percent elongation of continuous- but not of batch-cast films. This may be due to the fact that continuous-cast films had a higher density than the batch-cast films. Tomato films without carvacrol were thinner than the carvacrol-containing films. The amount of carvacrol in the films did not affect thickness.

**Table 4—Antibacterial effect after exposure of *E. coli* O175:H7 to tomato films with different concentrations of carvacrol for 24 and 48 h.**

| Casting method | Carvacrol concentration (% w/w) | Inhibition zone after 24 h incubation (mm$^2$) | Inhibition zone after 48 h incubation (mm$^2$) |
|---|---|---|---|
| Batch-cast | 0 | 0 | 0 |
| | 0.5 | 0 | 0 |
| | 0.75 | $14.1 \pm 6.3$ | $12.0 \pm 6.0$ |
| | 1.0 | $43.4 \pm 8.2$ | $37.8 \pm 16.2$ |
| Continuous-cast | 0 | 0 | 0 |
| | 0.5 | 0 | 0 |
| | 0.75 | $7.6 \pm 3.2$ | $10.5 \pm 4.2$ |
| | 1.0 | $20.7 \pm 6.4$ | $26.9 \pm 8.2$ |

**Film color.** Two-way ANOVA indicates that (1) $L^*$ and $b^*$ values of the films decreased with carvacrol concentration; (2) casting methods did not affect $L^*$ and $a^*$ values; and (3) batch-cast films had lower $b^*$ values than the continuous-cast films (Table 3). Generally, the effect of increased carvacrol concentration on color parameters was more pronounced in films prepared in batches under the biohood than those continuously cast in the Labcoater.

**Comparison of batch and continuous casting of films.** The 2 methods we used to prepare the films deserve additional comments. Both methods started with the same solution. For the batch method, the tomato puree was poured onto a glass plate with a Mylar coating placed over the top. A drawdown bar with a thickness of 45 mil was used to draw the puree down the plate. The puree was then cast at an even 45 mil and air dried in a biohood with a small amount of airflow over the top for 12 to 16 h at 23 to 27 °C.

For the continuous method, the puree was poured onto a continuously moving Mylar sheet. It was then passed through an infrared heater and then through an oven with a continuous airflow perpendicular to the film surface. This was followed by exposure to the ambient air, where it was cooled and wound on a roller.

The use of the casting method to prepare films may have advantages over the batch process in some applications for the following reasons. Films can be prepared in minutes rather hours, and the method requires less space and labor, allows the use of heat-sensitive solutions to make the films, and may be more suitable for large-scale commercial use.

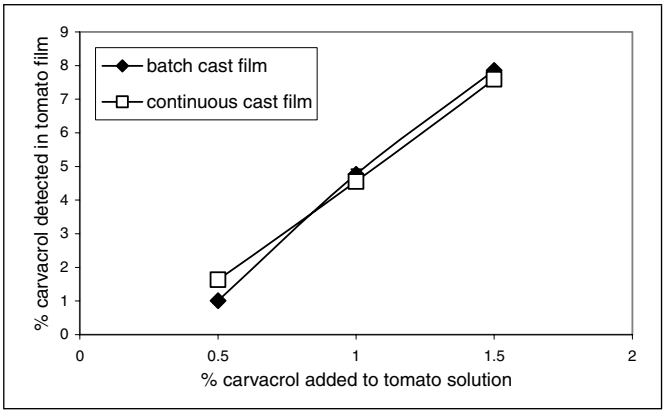



**Figure 3—Relationship between carvacrol levels in films made by the 2 casting methods (as determined by HPLC) and the amount of carvacrol originally added to the tomato film solutions.**

## Antimicrobial activities of films

**Relationship of carvacrol concentration in films to antimicrobial activities.** *E. coli* O157:H7 grew normally on agar plates with films lacking carvacrol incubated at 35 °C for 24 or 48 h. By contrast, no growth was observed on the plates around the film discs containing 0.75% or 1.0% carvacrol (Figure 1 and Table 4). The extent of bacterial growth inhibition increased as the percent carvacrol in the films was increased. Films prepared with 0% and 0.5% carvacrol did not inhibit growth of the bacteria.

Table 4 shows that, although not statistically different, films prepared with 0.75% carvacrol by the batch cast method induced a greater inhibition ($14.1 \pm 6.3$ mm$^2$ after 24 h incubation) than the corresponding films prepared by the continuous casting method ($7.6 \pm 3.2$ mm$^2$). The same trends are apparent for films prepared with 1% carvacrol ($43.4 \pm 8.2$ compared with $20.7 \pm 6.4$ mm$^2$). This behavior could be related to a slight reduction of carvacrol due to evaporation due to high temperature casting that also increased film density, resulting in more resistance for carvacrol diffusion to the agar media. The inhibition zones induced by films with 0.75% or 1.0% carvacrol on the Petri plates after 24-h incubation were similar to those observed following incubation for 48 h.

**Relationship between carvacrol content in film forming solutions and antimicrobial activities of films.** Based on the data in Figure 3, the following equations were developed to describe the

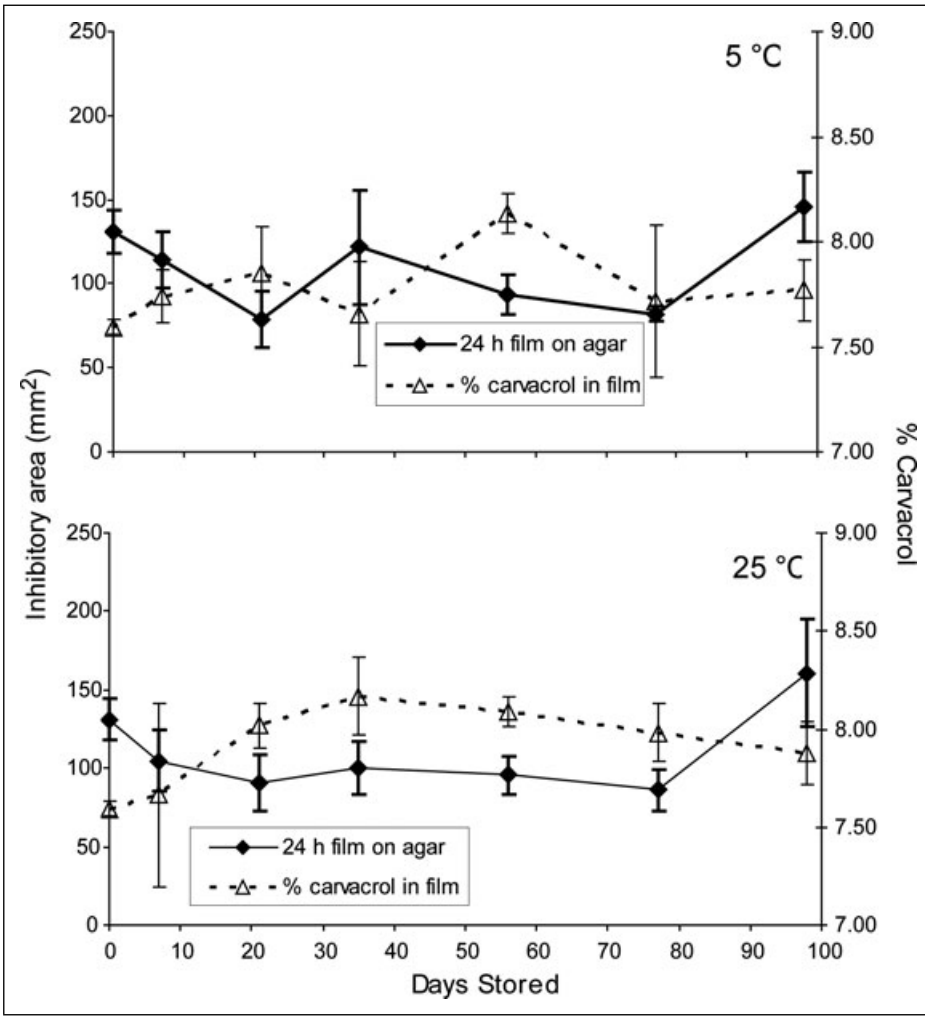



**Figure 4—Effect of storage of continuous casted films at 5 °C and 25 °C during 98 d on the antimicrobial activity (inhibitory zones) and carvacrol concentration of tomato films with 1.5% carvacrol added.**

linear relationship of carvacrol concentration in film forming solutions and final dried cast films:

$$B = 6.844c - 2.300 \ (n = 3; r^2 = 0.9984) \quad (2)$$

$$C = 5.965c - 1.374 \ (n = 3; r^2 = 0.9999) \quad (3)$$

where $B$ = % carvacrol in dried batch cast tomato films; $C$ = % carvacrol in dried continuous cast tomato films; $c$ = % carvacrol in tomato-film-forming solution; $r^2$ = correlation coefficient.

Based on these equations and the data in Table 4, we conclude that the minimum amount of carvacrol to be added to the tomato solution to ensure effective antibacterial activity is 0.75% for continuous and batch cast films. These concentrations of added carvacrol will ensure a minimum effective antimicrobial concentration of 3.5% final carvacrol in the films dried by the 2 casting methods.

**Relationship between storage of films and antimicrobial activities.** The tomato films with 1.5% carvacrol added were stored in temperature and humidity controlled incubators on open shelves. The carvacrol concentrations determined by HPLC with 50% ethanol extracts of tomato films made by continuous casting did not change significantly during storage of the tomato films for up to 3 mo at 5 and 25 °C (Figure 4). There were variations in carvacrol concentration during storage but the trend was not definitive. A possible explanation for the apparent increase of carvacrol concentration during storage is that the ratio of carvacrol to the weight of the film increased due to evaporation of water from the films during storage. The same pattern was observed with films made by the batch process (results not shown). The inhibition areas on Petri plates induced by the tomato films with 1.5% carvacrol were not affected by storage temperatures at 5 and 25 °C. However, the inhibition area was significantly higher ($P < 0.01$) for batch-cast films than continuous-cast films with this same formulation stored for 98 d at either 5 or 25 °C. Film weights did not change significantly following storage for up to 98 d (results not shown).

## Conclusions

The results of the present study show that carvacrol-containing tomato-based edible films inactivated the virulent pathogen *E. coli* O157:H7, that the inactivation was related to carvacrol levels in films determined by HPLC, that carvacrol in the films was stable during storage at 2 temperatures over a period of 98 d, and that films prepared by continuous casting are preferable for large-scale use than those prepared by batch casting. These observations facilitate optimizing effective levels of the antimicrobial in tomato films against *E. coli* and possibly other pathogens. Studies are currently under way to test the effectiveness of fruit and vegetable films against contaminated meat (Ravishankar and others 2008b).

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